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Phylogenetic and Biogeographic Relationships among North American and Hawaiian *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) Based on Chloroplast *rps4* and *rps4-trnS* Intergenic Spacer Sequences

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Abstract.—Nucleotide sequences encompassing the chloroplast *rps4* gene and the *rps4-trnS* intergenic spacer were obtained for several specimens representing North American and Hawaiian members of *Pteridium aquilinum* (ingroup), as well as *Pteridium esculentum* (outgroup). Nucleotide divergence between ingroup bracken taxa was low. The *rps4-trnS* intergenic spacer contained indels distinguishing *P. aquilinum* and *P. esculentum*. Phylogenetic analyses and a haplotype network recognized two major groups within Northern American bracken that are divided along both genetic and geographic lines. The Hawaiian var. *decompositum* and the western North American var. *pubescens* share a similar chloroplast genome and grouped together. Despite morphology and geographical distribution, sequences for var. *feei* and eastern North American var. *latiusculum* were very similar and grouped together. Sequence data could not distinguish eastern North American var. *latiusculum* and the southeastern North American var. *pseudocaudatum*. Haplotype and biogeographic analyses suggest a most recent common eastern North American ancestor for the ingroup.

Key Words.—Biogeography, Dennstaedtiaceae, Hawaii, North America, phylogenetics, *Pteridium*, *rps4*

Despite the fact that the bracken fern is one of the world's most common plants and has been widely studied, many aspects of its systematics are still poorly understood. While a number of systematic treatments have relied heavily on morphological variation, bracken morphology is highly problematic and can vary both within and among populations, as well as among different taxa. This problem was acknowledged by Tryon (1941), who pointed out that diagnostic characters were often variable within taxa and were not constant across the genus. Tryon (1941) recognized a single species, *Pteridium aquilinum* (L.) Kuhn, which he divided into two subspecies and twelve varieties.

To get around the systematic problems presented by morphology, many have employed molecular approaches in order to resolve better the relationships within and among bracken taxa. These have included the use of isozymes (e.g., Korpelainen, 1995; Speer *et al.*, 1999), restriction site analysis (e.g., Thomson *et al.*, 1995; Wolf *et al.*, 1995), DNA fingerprints (e.g., Thomson *et al.*, 2005) and DNA nucleotide sequence data (e.g., Speer, 2000).

One consistent problem in many of these studies is the lack of regular, discernible infraspecific variation. The genetic relationships between many taxa tend to be poorly understood. While Speer *et al.* (1999) conducted a population genetic study using isozymes to examine the eastern North

American *P. aquilinum* var. *latiusculum* (Desv.) Underw. and *P. aquilinum* var. *pseudocaudatum* (Clute) Heller, no comparable studies have been conducted within the western North American *P. aquilinum* var. *pubescens* Underw. or between it and either of the two eastern varieties. Furthermore, the taxonomic connection between the Hawaiian endemic *P. aquilinum* var. *decompositum* (Gaudich.) R.M. Tryon and the other Northern Hemisphere taxa is poorly understood.

The systematic relationships among five North American and Hawaiian bracken taxa were examined using chloroplast *rps4* plus *rps4-trnS* intergenic DNA sequences. The objectives of this study were 1) to determine if phylogenetic relationships among North American bracken are congruent with previous morphological treatments of the genus, 2) to use chloroplast gene sequences to assess the systematic affiliations of varieties *pseudocaudatum*, *pubescens*, *feei*, and *latiusculum*, and 3) to investigate the relationship of the Hawaiian var. *decompositum* to the North America taxa. Such information is not only necessary for an adequate understanding of *Pteridium* systematics, but is of considerable importance for understanding bracken biogeography.

Materials and Methods

Taxon selection.—Several aspects of *Pteridium* systematics are quite controversial, though most of the current taxonomic controversy involves the Eurasian taxa, with some of Tryon's (1941) varieties being divided into as many as three or four different taxa in some treatments (e.g., Page, 1995; Bridges *et al.*, 1998; Ashcroft and Sheffield, 1999; Shorina and Perestoronina, 2000; Thomson, 2004; Thomson *et al.*, 2005). To avoid some of the more confusing facets of bracken taxonomy, none of these taxa were included in this study. In contrast, the North American and Hawaiian taxa examined here are considerably less controversial and are recognized by virtually all authorities (e.g., Lellinger, 1985; Thomson, 2004).

Thirteen bracken specimens representing all five North American and Hawaiian varieties (*sensu* Tryon) were used in this study (Table 1). These also included two downloaded GenBank accessions for var. *latiusculum* (GenBank AY626796) and Mexican *P. aquilinum* var. *feei* (Schaffner ex Fée) Faull (GenBank AY690319). In addition, two *P. esculentum* (Forst.) Nakai (= Tryon's *P. aquilinum* var. *esculentum* (Forst.) Kuhn) specimens were used as the outgroup for the phylogenetic analysis. In contrast to the ingroup, *P. esculentum* is a southern hemisphere bracken. Tryon (1941) divided all bracken into two subspecies. Accordingly, the ingroup taxa belong to subsp. *aquilinum* (= Tryon's subsp. *typicum*), while the outgroup taxon was placed in subsp. *caudatum* (L.) Bonap. With the exception of var. *feei*, each taxon is represented by two or more specimens from different geographic locations.

DNA extraction, PCR, and sequencing.—Total genomic DNA was extracted from the frond material using the Doyle and Doyle (1987) method. One hundred ng of DNA was used in each 100 μl PCR reaction mixture. Individual reaction mixtures were amplified using forward (5′-ATGTCCCGTTATCGAG-

TABLE 1. Sources of ingroup and outgroup material providing *rps4* plus *rps4-trnS* intergenic sequences.

TAXON	ORIGIN	COLLECTION	GENBANK
Ingroup:			
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield 30**	DQ426652
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield H2**	DQ426658
P. aquilinum var. *decompositum*	Hawaii, USA	*E. Sheffield K3**	DQ426650
P. aquilinum var. *decompositum*	Hawaii, USA	*W. Speer 276*	AF197100
P. aquilinum var. *feei*	Veracruz, Mexico	*K. Mehltreter 1064*	AY690319**
P. aquilinum var. *latiusculum*	New Hampshire, USA	*Haufler & Haufler s.n.*	DQ486983
P. aquilinum var. *latiusculum*	New Jersey, USA	*R. Moran s.n.*	DQ426651
P. aquilinum var. *latiusculum*	New York, USA	*P.G. Wolf s.n.*	DQ486979
P. aquilinum var. *latiusculum*	Michigan, USA	*NSW420310*	AY626796**
P. aquilinum var. *pseudocaudatum*	Florida, USA	*E. Sheffield 31**	DQ416774
P. aquilinum var. *pseudocaudatum*	S. Carolina, USA	*Speer and Speer s.n.*	AF197101
P. aquilinum var. pubescens	Utah, USA	*W. Speer* 242	AF197095
P. aquilinum var. pubescens	California, USA	*P.G. Wolf* 652*	DQ426657
Outgroup:			
P. esculentum	Australia	*E. Sheffield* 105*	DQ426655
P. esculentum	Tasmania	*E. Sheffield* 115*	DQ486984

*DNA samples with collection information supplied by P. G. Wolf (USU).
**Sequence author: J. A. Thomson (National Herbarium of NSW). Sequence downloaded from GenBank

GACCT-3′) and reverse (5-TACCGAGGGTTCGAATC-3′) primers. Thermocycling involved heating the PCR reaction mixtures to 95°C for 5 min., followed by 30 cycles of 95°C (1 min), 42°C (1.5 min) and 72°C (1 min), concluded by a final extension of 72°C for 10 min., and storage at 4°C in a GeneAmp® PCR System 2400 (Perkin-Elmer, Norwalk, CT, USA). A Wizard® PCR Prep Purification System (Promega, Madison, WI, USA) was used to purify the PCR products prior to sequencing.

All sequencing reactions used BigDye™ Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA, USA). Using a GeneAmp® PCR System 2400 (above), sequencing reactions were heated to 96°C for 1 s, followed by 30 cycles of 96°C (1 s), 47°C (5 s) and 60°C (4 min), and then stored at 4°C. Reactions were cleaned using sephadex columns, loaded onto an acrylamide gel, and electrophoresed on an ABI Prism® 377 DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequences were aligned with Sequencher™ 3.1.RC4 (Gene Codes Corporation, Ann Arbor, MI, USA) using the "dirty data" algorithm with default alignment settings (80% minimum match, 20 bp minimum overlap). Sequence editing was done by sight inspection of sequences. All 13 sequences obtained in this fashion were submitted to GenBank.

Phylogenetic analysis.—Data matrices of aligned sequences were first assembled in MacClade 3.07 (Maddison and Maddison, 1992) and then saved as Nexus Files. Maximum Parsimony (MP) analyses were conducted using

PAUP* 4.0b10 (Swofford, 1999) with default settings, including ACCTRAN optimization. Analyses were conducted a) with indels coded (Simmons and Ochoterena, 2000), but gaps otherwise treated as missing data and b) with gaps treated as a fifth base. The data were unordered and equally weighted. *Pteridium esculentum* was designated as the outgroup taxon and trees were rooted by making the outgroup a monophyletic sister group to the ingroup. The heuristic algorithm was used in tree construction. For tree evaluation, the following statistics were compiled: consistency index (CI), the retention index (RI), the number of most parsimonious trees, tree length, and the number of parsimony informative characters. A 50% majority rule consensus tree was generated. Bootstrapping was also performed using a heuristic search with 100 random addition sequences with simple-addition sequence and TBR swapping. Because of the perceived low levels of nucleotide diversity among the *Pteridium* specimens, potential phylogenetic signal was evaluated in PAUP* using 1) the Evaluate Random Trees option (1,000 random trees) to obtain a *g1* statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992) and 2) a Permutation Tail Probability (PTP) test for randomness (lack of phylogenetic signal) in the data (Faith and Cranston, 1991). Because the sequence alignment and analysis indicated low levels of nucleotide divergence, PAUP* was also used to construct a matrix of pairwise distances (uncorrected "p") in order to assess and quantify divergence within and between the different *Pteridium* groups.

Prior to the maximum likelihood (ML) analyses, the computer program ModelTest 3.04 (Posada and Crandall, 1998) was employed to determine the appropriate substitution model for ML, which in this case was the Hasegawa-Kishino-Yano 1985 (HKY85). The ML analysis was conducted using PAUP* 4.0b10 (Swofford, 1999) using the HKY85 model with the Empirical Base Frequencies (A = 0.31043, C = 0.18566, G = 0.18266, T = 0.32125) option selected. The Ti/Tv (transition/transversion) ratio was set to 1.46667. For Among-Site Rate Variation, Equal Rates For All Sites was selected and the Proportion Of Invariable Sites set to zero. A heuristic search was used in tree construction. Bootstrapping was also performed.

Haplotype analysis.—In order to further evaluate relationships within this group of closely related bracken ferns, haplotype networks were produced using the computer programs TCS 1.21 (Clement *et al.*, 2000) and Network 4.500 (available at www.fluxus-engineering.com). TCS 1.21 uses a statistical parsimony approach. Because of the low level of nucleotide variation among sequences, haplotype connectivity was left at the default setting of 95% parsimony. The median-joining method (Bandelt *et al.*, 1999) was employed in Network 4.500.

Biogeographic analyses.—Putative ancestral distributions were reconstructed using the DIVA program, version 1.1 (Ronquist, 1997). Quartet puzzle (QP) analyses were performed in PAUP* to produce completely bifurcating trees, as required by DIVA. To do this, OTUs were limited to one representative for each of the five haplotypes determined by TCS (see Results), plus an outgroup (*P. esculentum*) haplotype. The analyses were conducted for each of the three optimality criteria available in PAUP*. Based on the ModelTest results, the

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OUTGROUP:          880                                                                  967
esculentum         TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCTAGTCTTTCTTTCTCAAATCGGTAAATTAGCGAGATTTTCAAAA

INGROUP
latiusculum        TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA
pseudocaudatum     TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA
pubescens          TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA
decompositum       TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA
feei               TCTGTTTTGGTTTGGACGGCTTTATTTGAAACCCAAGTCT-TTCTTTCT----CAAATCGGTAAATTAGCGAGATTTTCAAAA
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FIG. 1. Alignment of sequences by taxon in the indel region of the chloroplast *rps4-trnS* intergenic spacer.

HKY85 setting was used for the ML and the distance (minimum evolution) QP analyses. Following the QP analyses, a haplotype distribution data matrix was constructed using MacClade 3.07. After the data matrix was made, the "Tree Window" option was used to produce trees with the same topologies as the QP trees. Each tree plus the data matrix was saved as a single Nexus file in MacClade. To run DIVA 1.1 on a PC, these files required some minor editing. Five distribution areas were identified: eastern North America (A), western North America (B), Hawaii (C), Mexico (D), and Oceania (E). All DIVA analyses were optimized using the "maxareas=2" option.

RESULTS

Sequence alignment.—Sequences covering both the chloroplast *rps4* gene and the *rps4-trnS* spacer were produced for the 13 *Pteridium* specimens. Sequences 954 bp in length were generated for bracken described by Tryon (1941) as varieties *latiusculum*, *pseudocaudatum*, *pubescens*, and *decompositum*. For the outgroup *P. esculentum*, sequences were 959 bp long. The aligned portions of the downloaded var. *latiusculum* and var. *feei* sequences were 508 bp and 721 bp, respectively.

Indel sites were found within the *rps4-trnS* spacer to be informative and separated the outgroup *P. esculentum* from the ingroup taxa (Fig. 1). These consisted of a single nucleotide site and a nearby multiple nucleotide indel (-TTCT-) in a tandem repeat region in the *rps4-trnS* intergenic spacer.

Phylogenetic analyses.—The MP and ML analyses had comparable results and produced trees with identical topologies. There was an average pairwise distance (uncorrected "p") of 0.01370 between the outgroup and the ingroup taxa (Table 2). Most of the nucleotide variance within the ingroup was among the eastern North American bracken, which had a mean distance of 0.00114.

There were 22 variable characters, of which 18 were parsimony informative. Of these, 17 separated *P. aquilinum* (ingroup) from *P. esculentum* (outgroup). A single purine transition (G ↔ A) split the ingroup along more or less geographical lines, and the Mexican var. *feei* joined with the eastern North American plants. There was no difference between analyses with indels coded

TABLE 2. Summary of pairwise distances (uncorrected "p") for *Pteridium* taxa.

Group	Range	Mean	(S.D.)
All bracken	0.00000–0.01531	0.00444	(0.00547)
Outgroup	0.00000	0.00000	—
Ingroup	0.00000–0.00419	0.00137	(0.00111)
Eastern North America (ENA)	0.00000–0.00388	0.00114	(0.00125)
Western North America-Hawaii (HWN)	0.00000–0.00105	0.00035	(0.00035)
Outgroup v. Ingroup	0.01257–0.01531	0.01370	(0.00082)
ENA v. HWN	0.00105–0.00419	0.00192	(0.00089)

and those with gaps treated as a fifth base. Six equally parsimonious trees were produced (length = 22 steps, CI = 1.000, RI = 1.000).

Bootstrap support between the ingroup and outgroup was 100%. Bracken from western North America and Hawaii grouped together in a weakly supported (61%) clade, while var. *feei* and the eastern North American *Pteridium* formed a large polytomy (Fig. 2). Beyond these considerations, it was not possible to distinguish infraspecific taxa by the sequence data.

Despite the relatively low levels of observed nucleotide divergence for the sequences obtained, a value of $g1 = -3.859$ was obtained using PAUP*. This was interpreted as signifying that the data matrix has a strongly nonrandom structure (skewness), which is an indication that it may contain significant phylogenetic signal. This was supported by the PTP test, which indicated significant nonrandom structure ($P = 0.01$) in the data.

A single maximum likelihood tree was produced (−ln = 1415.1850), which was identical with the MP 50% majority-rule consensus tree. Bootstrap values obtained by ML were comparable to those acquired by MP, with support between ingroup and outgroup at 100% and the western North America-Hawaii clade weakly receiving 67% support.

Haplotype analysis.—TCS collapsed the 13 ingroup sequences into five haplotypes that were divided into two major groups (Fig. 3, Table 3). Varieties *feei*, *latiusculum*, and var. *pseudocaudatum* comprised the first group (ENA), while the second (HWN) was composed of the Hawaiian var. *decompositum* and the western North American var. *pubescens* (Table 3). Within the ENA group were three haplotypes. ENA-2 and ENA-3 were represented by the var. *latiusculum* sequences from New York and New Jersey, respectively. All other eastern bracken sequences (including the one for var. *feei*) collapsed to form haplotype ENA-1. The HWN-1 haplotype accounted for the majority of sequences from varieties *decompositum* and *pubescens*, with a single Hawaiian bracken sequence (GenBank DQ426658) comprising the HWN-2 variant. As in the phylogenetic analyses, the demarcation of haplotypes tended to follow geography more than recognized infraspecific taxonomy. The statistical parsimony (TCS 1.21) and median-joining (Network 4.500) networks were identical.

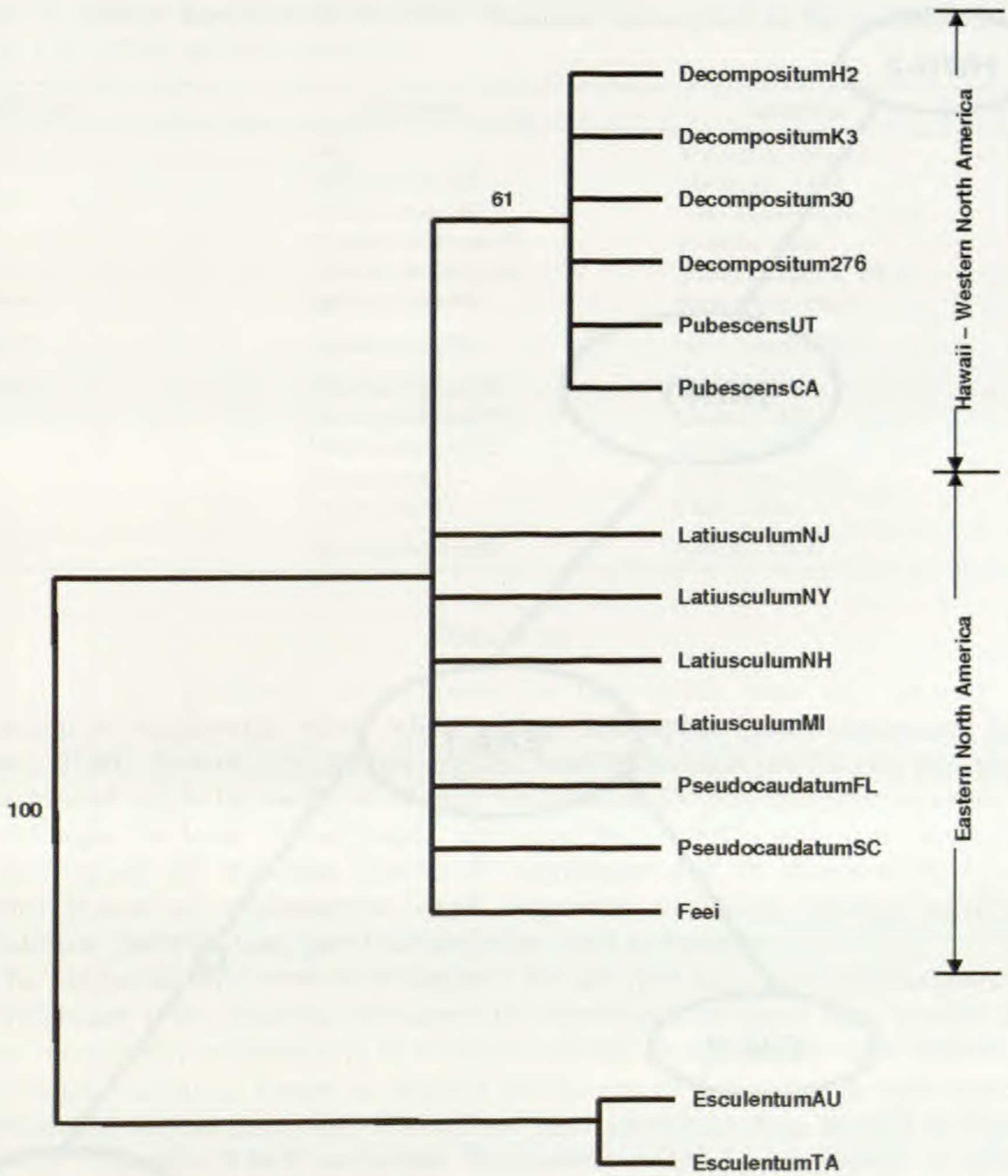

FIG. 2. 50% majority consensus tree of six equally parsimonious trees (Length = 22 steps, CI = 1.000, RI = 1.000) produced from 15 *rps4* plus *rps4-trnS* intergenic spacer sequences. Bootstrap percentages are shown. The maximum likelihood tree had an identical topology and comparable bootstrap support values. See Table 1 for specimen and collection information.

Biogeographic analyses.—The resulting QP trees differed only in their determination of which of the three ENA haplotypes was the most basal within the ingroup. Otherwise, they had identical topologies. Despite these minor disparities, the DIVA outcomes were identical in all three cases. The QP tree using the distance optimality criterion is shown in Fig. 4 because its topology was interpreted as being the most consistent with the phylogenetic analyses. The results indicated that the most recent common ancestor (MRCA) for the

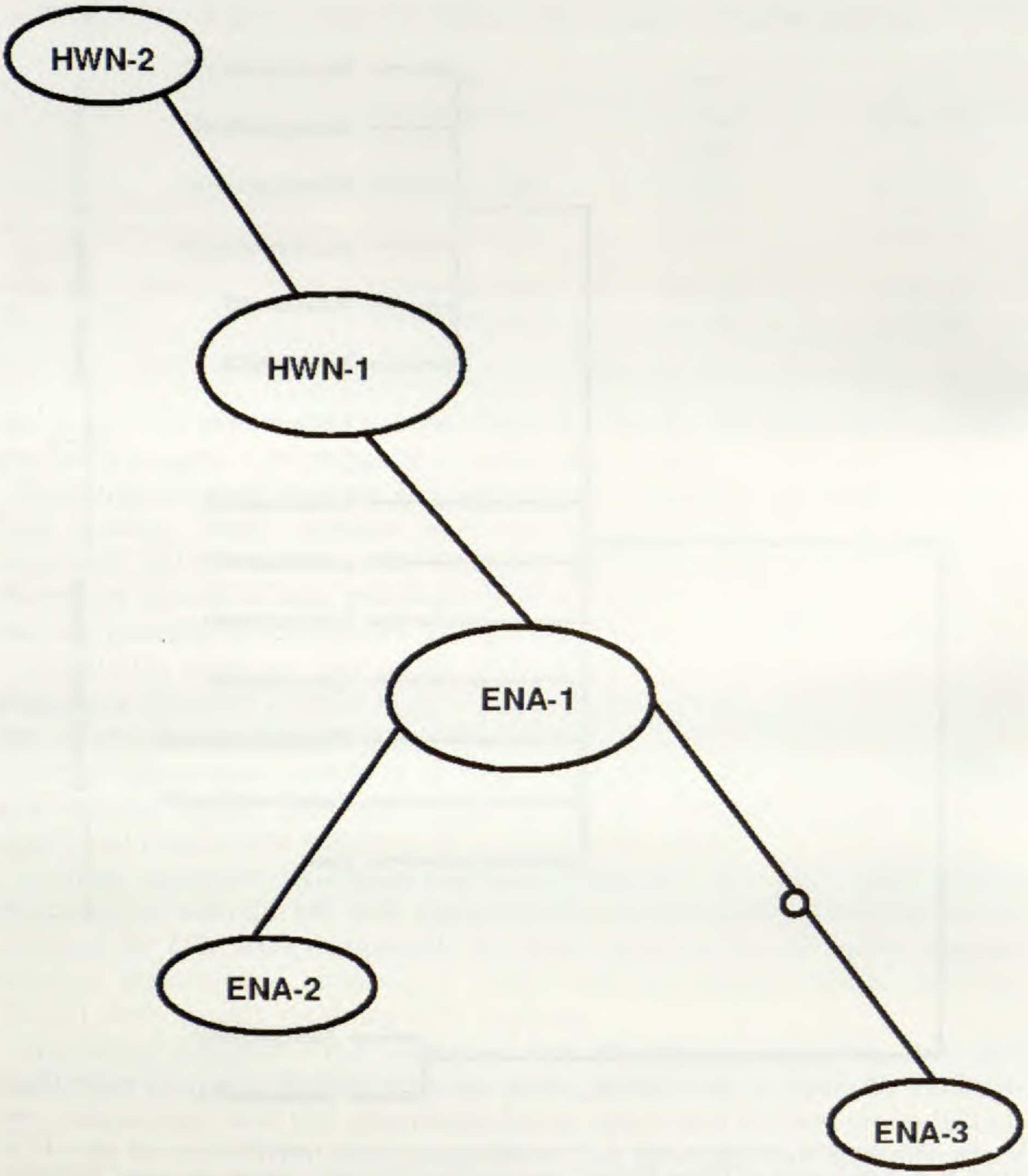

FIG. 3. Haplotype network for North American bracken using both statistical parsimony and median-joining approaches. ENA = Eastern North America and HWN = Hawaii–Western North America. Each line segment represents a single nucleotide difference. See Table 3 for a listing of haplotypes according to specimen/taxon.

ingroup had an eastern North America (A) distribution (Fig. 4). Assuming the Oceanic *P. esculentum* as the outgroup, the optimal reconstruction assumes four dispersals to account for the ingroup distribution. DIVA indicated a Hawaiian ancestor for the two HWN haplotypes, though the haplotype analyses suggested another possibility (see Discussion).

TABLE 3. Bracken haplotypes by specimen. Specimens are as given in Fig. 2. Please consult Table 1 for further specimen information.

Haplotype	Specimen	Location
ENA-1	Feei	Veracruz, Mexico
	LatiusculumMI	Michigan, USA
	LatiusculumNH	New Hampshire, USA
	PseudocaudatumFL	Florida, USA
	PseudocaudatumSC	South Carolina, USA
ENA-2	LatiusculumNY	New York, USA
ENA-3	LatiusculumNJ	New Jersey, USA
HWN-1	Decompositum30	Hawaii, USA
	Decompositum276	Hawaii, USA
	DecompositumK3	Hawaii, USA
	PubescensCA	California, USA
	PubescensUT	Utah, USA
HWN-2	DecompositumH2	Hawaii, USA

DISCUSSION

One of the problems encountered in this study was the paucity of informative nucleotide sites when using chloroplast gene sequences (see Speer, 2000). Several chloroplast regions were examined previously and were determined not to be useful or of very limited use for phylogenetic analysis at an infraspecific level. For example, although Wolf (1997) used *atpB* sequences to distinguish the bracken species *P. aquilinum* and *P. esculentum*, Speer (2000) found no informative *atpB* sequence variation among varieties *aquilinum*, *latiusculum*, *pseudocaudatum*, and *pubescens*.

The extremely low level of divergence for the *rps4* plus *rps4-trnS* sequences is congruent with previous infraspecific treatments of these taxa, which are often recognized as belonging to a single species: *P. aquilinum*. The pattern of nucleotide variation, however, did not follow any of the earlier morphological taxonomies. In contrast, the chloroplast gene sequence data tended to lump together Tryon's (1941) varieties. Consistent with Speer (2000), a close phylogenetic relationship was observed for varieties *latiusculum*, *pseudocaudatum*, and *pubescens*. Based on the indel patterns found in the *rps4-trnS* intergenic spacer, all ingroup sequences belong to the "*latiusculum*" haplotype group described by Speer (2000)(see also Speer *et al.*, (2001) and Speer *et al.*, (2002)). This is also designated by Thomson *et al.* (2005) as the bracken fern "Haplotype A" group.

Hawaiian-Western North American clade.—The Hawaiian var. *decompositum* and the western North American var. *pubescens* were united in a single clade. A single A↔G transition unites the Hawaiian and western North American bracken (G) and separates them from the eastern plants (A).

Although Fosberg (1948) determined that most Hawaiian natural plant populations were southeast Asian in origin, he identified a small minority that was most likely of North American origin. North American–Hawaiian

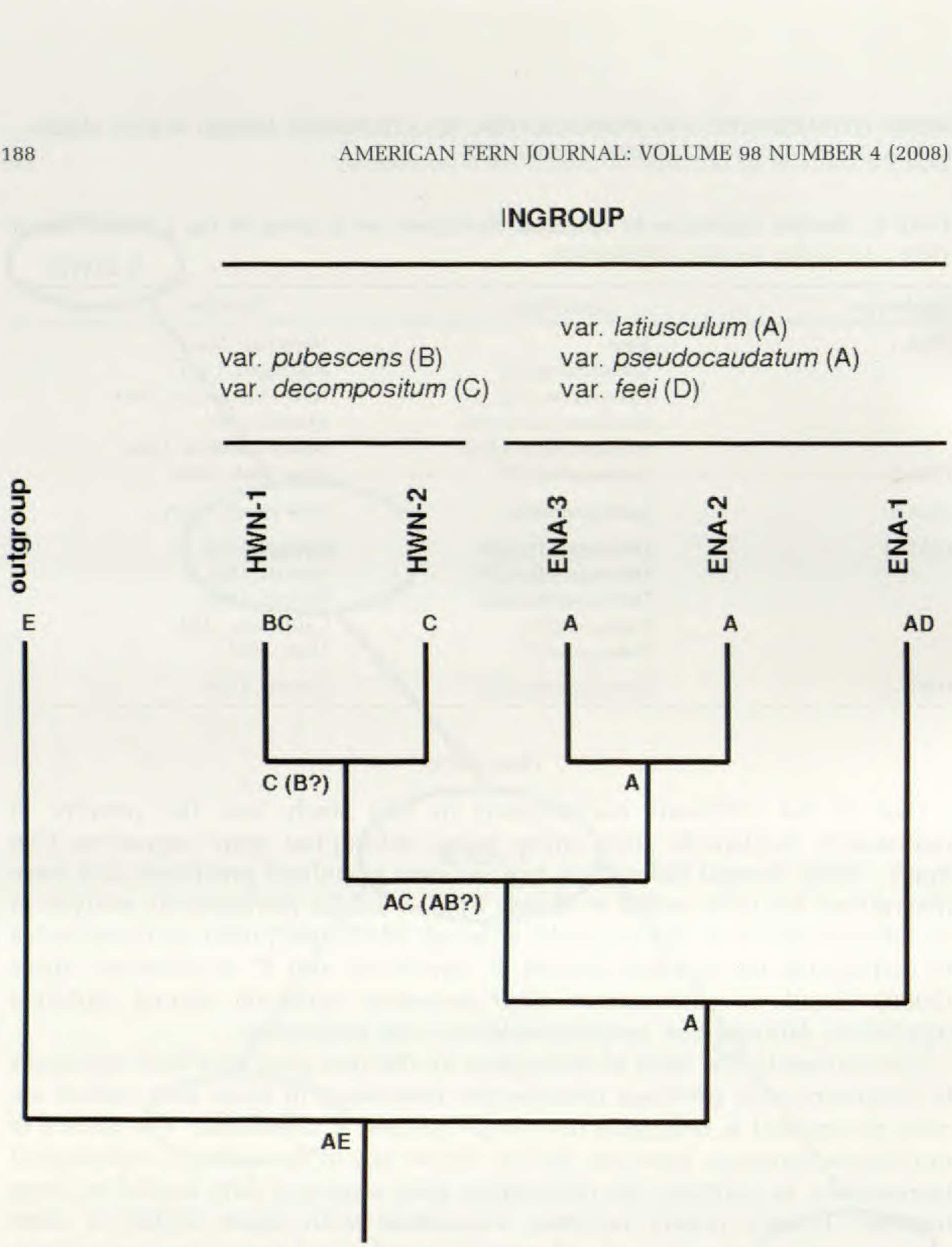

FIG. 4. Putative optimal reconstruction of the ancestral distributions of North America *Pteridium* using the program DIVA. Five distribution areas were identified: A = eastern North America; B = western North America; C = Hawaii; D = Mexico; E = Oceania. Ancestral distributions are given at internal nodes.

relationships are known to exist for a number of plant taxa, including the fern genus *Asplenium* (Ranker *et al.*, 1994) and the angiosperm genera *Sanicula* (Vargas *et al.*, 1998) and *Stachys* (Lindqvist and Albert, 2002). Tryon (1941) felt that var. *decompositum* had a North American relationship and suggested a close relationship with both var. *pubescens* and var. *feei*, although the cpDNA sequence evidence here favors the former taxon but not the latter. Sheffield *et al.* (1995) produced isozyme data that indicated a potential relationship

between Hawaiian and North American bracken, although some differences in isozyme profiles were observed between Hawaiian and North American bracken populations. These findings, however, differ with the DNA fingerprint studies of the genus (Thomson, 2000; Thomson, 2004), which tend to indicate that var. *decompositum* shares some common band profiles with the southeast Asian *P. aquilinum* var. *wightianum* (J. Agardh) Shieh (= *P. revolutum* (Blume) Nakai) and/or east Asian representatives of var. *latiusculum* (= *P. aquilinum* subsp. *japonicum* (Nakai) A. Löve & D. Löve). Considering that the Hawaiian flora tends to be a combination of southeast Asian and North American elements (Fosberg, 1948), it is entirely possible that the Hawaiian var. *decompositum* is a hybrid between eastern Asian and western North American bracken.

Because of their volcanic origin and central Pacific location, the Hawaiian islands appear to always have been very isolated from the larger continental land masses, as well as islands of continental origin (Wilson, 1963; Clague and Dalrymple, 1987). Because of Hawaii's considerable geographic isolation, long distance dispersal would have to account for a substantial portion of the current Hawaiian biota. Long distance dispersal can occur very easily in many pteridophyte species through the production of small, wind-borne spores (Tryon, 1970).

Geiger *et al.* (2007) identified four climate and weather based mechanisms that could promote long distance spore dispersal to Hawaii and account for the current Hawaiian pteridophyte flora: 1) northern neotropical jetstream moving from the southeast Asia, 2) trade winds from Central and western North America, 3) storms from southern Mexico and Central America, and 4) the combined influence of the Intertropical Convergence Zone and Hadley Cell air movements, which could move spores from the South Pacific region. Two of these mechanisms are clearly relevant to the current discussion. The northern tropical jetstream could move spores of var. *wightianum* from southeast Asia to Hawaii, while the northern trade winds could disperse var. *pubescens* spores from northwestern Mexico. It is, therefore, possible that spores from both regions could have ended up in Hawaii, giving rise to var. *decompositum*. While this hypothesis requires further examination, it does harmonize the findings of Thomson (2000) and the present study.

Eastern North American Pteridium.—The close taxonomic relationship of var. *feei* with the other North American taxa was previously observed by Thomson *et al.* (2008), using a combined morphometric, DNA fingerprint (AP-PCR), and cpDNA approach. The haplotype analysis of the present study not only confirms this finding, but further clarifies this relationship by showing that var. *feei* is more closely related to bracken in eastern North America than it is to those in western North America and Hawaii. This plant has a southern Mexican and Central American range (Tryon, 1941; Smith, 1993), which is well within the much larger distributions of *P. caudatum* (L.) Maxon and *P. arachnoideum* (Kaulf.) Maxon, both of which Tryon (1941) treated as varieties in the southern subsp. *caudatum*. For most of its distribution, it falls into the

same range of longitude as the more northern var. *latiusculum* and var. *pseudocaudatum*.

Throughout much of the northern end of its range, var. *feei* is 700 miles or less from the southern end of the overlapping var. *latiusculum* and var. *pseudocaudatum* distributions in the southeastern United States, but is even closer to disjunct populations of var. *latiusculum* in the Sierra Madre Oriental mountains of northeastern Mexico (Tryon, 1941). Since bracken spores could be easily dispersed over such distances, it is possible that var. *feei* may have started as a southern disjunct of one of these two more northern taxa. Alternatively, it could have originated from plants that became isolated as the distribution of most North American bracken gradually shifted northward following the Pleistocene. Population genetics investigations are needed to determine if there is evidence to support either of these hypotheses, as well as the possibility of gene flow between bracken in these geographical regions.

This study supports the close genetic relationship between varieties *latiusculum* and *pseudocaudatum* as described in the isozyme research of Speer *et al.* (1999) and substantiated by the morphometric and AP-PCR analyses of Thomson (2000) and the chloroplast DNA study of Speer (2000). Speer and Hilu (1999) cite personal communication from Tryon describing var. *pseudocaudatum* as a "weak variety" due to the strong morphological similarities between it and var. *latiusculum*. The two var. *pseudocaudatum* specimens included in this inquiry had cpDNA sequences very similar to those found for var. *latiusculum*. There were no synapomorphies that united them into a distinct clade or distinguished them from the eastern North American var. *latiusculum*. Speer *et al.* (1999) found that these two geographically overlapping bracken taxa encompass a single uninterrupted gene pool. The isozyme and cpDNA evidence is consistent with the view that these are not two separate taxa, but a single bracken variety with northern (*latiusculum*) and southern (*pseudocaudatum*) morphotypes.

Ancestral distributions in North America.—The DIVA results support an eastern North American MRCA for varieties *latiusculum*, *pseudocaudatum*, *pubescens*, *decompositum*, and *feei*. Such an inference is compatible with the phylogenetic and haplotype analyses, though other interpretations are possible.

DIVA suggested that the ancestor for both var. *pubescens* and var. *decompositum* had a Hawaiian distribution. This would imply 1) migration from eastern North America to Hawaii and 2) then dispersal from Hawaii to western North America. While this scenario cannot be ruled out, the pattern of haplotype divergence (Fig. 3) suggested another possibility, with 1) movement from eastern North America into western North America and 2) a subsequent dispersal from western North America to Hawaii, which agrees with Geiger *et al.* (2007). It should also be noted that var. *latiusculum* (*sensu* Tryon) has an almost completely circumboreal distribution, being found throughout Eurasia and eastern North America. It is primarily in western North America that a gap is seen, with var. *pubescens* being found instead. Given their very similar morphologies (Tryon, 1941) and the minimal genetic divergence between

them, varieties *latiusculum* and *pubescens* do appear to be very closely related. In contrast to the situation with var. *pseudocaudatum*, however, the current geographical and molecular evidence favors a continued recognition of the western North American bracken as a distinct taxon, though at an infraspecific level.

It is becoming increasingly apparent that the morphological taxonomies of Tryon (1941) and others do not reflect accurately many of the systematic relationships within *Pteridium*. At the very least, a thorough re-examination of the morphological characters used to delineate bracken taxa is needed. While molecular sequence data have contributed to an improved understanding of *Pteridium* systematics, much work is still needed. It is anticipated that continuing work will answer many of the yet unresolved questions to provide a new and revised *Pteridium* taxonomy.

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LITERATURE CITED

ASHCROFT, C. J. and E. SHEFFIELD. 1999. Rejection of *Pteridium aquilinum* subspecies *atlanticum* (C. N. Page). Botanical Journal of the Linnean Society 130:157–170.

BANDELT, H-J., P. FORSTER and A. RÖHL. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution. 16:37–48.

BRIDGES, K. M., C. J. ASHCROFT and E. SHEFFIELD. 1998. Population analysis of the type localities of some recently recognised taxa of British *Pteridium* (Dennstaedtiaceae: Pteridophyta). Fern Gazette 15:205–213.

CLAGUE, D. A. and G. B. DALRYMPLE. 1987. The Hawaiian-Emperor volcanic chain. Pp. 5–84, in *Volcanism in Hawaii. U.S. Geological Survey Professional Paper 1350*, R. W. Decker, T. L. Wright and P. H. Stauffer, (eds.) Washington, D.C: United States Government Printing Office.

CLEMENT, M., D. POSADA and K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

DOYLE, J. J. and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

FAITH, D. P. and P. S. CRANSTON. 1991. Could a cladogram this short have arisen by chance alone? On permutation tests for cladistic structure. Cladistics 7:1–28.

FOSBERG, F. R. 1948. Derivation of the flora of the Hawaiian Islands. Pp. 107–119, in *Insects of Hawaii*, Vol. 1, E. C. Zimmerman, (ed.) Honolulu: University of Hawaii Press.

GEIGER, J. M. O., T. A. RANKER, J. M. RAMP NEALE and S. T. KLIMAS. 2007. Molecular biogeography and origins of the Hawaiian fern flora. Brittonia 59:142–158.

HILLIS, D. M. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. Pp. 278–294, in *Phylogenetic analysis of DNA sequences*, M. M. Myamoto and J. Craft, (eds.) New York: Oxford University Press.

HILLIS, D. M. and J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. Journal of Heredity 83:189–195.

KORPELAINEN, H. 1995. Mating system and distribution of enzyme genetic variation in bracken (*Pteridium aquilinum*). Canadian Journal of Botany 73:1611–1617.

LELLINGER, D. B. 1985. *A Field Manual of the Ferns and Fern-Allies of the United States and Canada.* Washington, D.C: Smithsonian Institution Press.

LINDQVIST, C. and V. A. ALBERT. 2002. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). American Journal of Botany 89:1709–1724.

MADDISON, W. P. and D. R. MADDISON. 1992. *MacClade: Analysis of Phylogeny and Character Evolution.* Sunderland, Massachusetts: Sinauer Associates.

PAGE, C. N. 1995. Structural variation in Western European bracken: An updated taxonomic perspective. Pp. 13–15, in *Bracken: An Environmental Issue*, R. T. Smith and J. A. Taylor, (eds.) Aberystwyth: International Bracken Group.

POSADA, D. and K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817–818.

RANKER, T. A., S. K. FLOYD, M. D. WINDHAM and P. G. TRAPP. 1994. Historical biogeography of *Asplenium adiantum-nigrum* (Aspleniaceae) in North America and implications for speciation theory in homosporous pteridophytes. American Journal of Botany 81:776–781.

RONQUIST, F. 1997. Dispersal–vicariance analysis: A new approach to the quantification of historical biogeography. Systematic Biology 46:195–203.

SHEFFIELD, E., P. G. WOLF and T. A. RANKER. 1995. Genetic analysis of bracken in the Hawaiian Islands. Pp. 29–32, in *Bracken: An Environmental Issue*, R. T. Smith and J. A. Taylor, (eds.) Aberystwyth: International Bracken Group.

SHORINA, N. I. and O. N. PERESTORONINA. 2000. Taxonomic studies of Russian bracken I: Taxonomy of *Pteridium* in territories of European Russia, Crimea and Caucasus. Pp. 48–51, in *Bracken Fern: Toxicity, Biology, and Control*, J. A. Taylor and R. T. Smith, (eds.) Leeds: International Bracken Group.

SIMMONS, M. P. and H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49:369–381.

SMITH, A. R. 1993. Phytogeographic Principles and Their Use in Understanding Fern Relationships. Journal of Biogeography 20:255–264.

SPEER, W. D. 2000. A systematic assessment of British and North American *Pteridium* using cpDNA gene sequences. Pp. 37–42, in *Bracken Fern: Toxicity, Biology, and Control*, J. A. Taylor and R. T. Smith, (eds.) Leeds: International Bracken Group.

SPEER, W. D. and K. W. HILU. 1999. Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). I. Morphological evidence. Systematic Botany 23:305–312.

SPEER, W. D., E. SHEFFIELD and P. G. WOLF. 2001. Relationships among Northern Hemisphere *Pteridium*: Some new perspectives. In: *Botany, Plants and People.* Albuquerque, NM: Botanical Society of America, 544.

SPEER, W. D., C. R. WERTH and K. W. HILU. 1999. Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence. Systematic Botany 23:313–325.

SPEER, W. D., C. R. WERTH, E. SHEFFIELD and P. G. WOLF. 2002. North American *Pteridium*: Insights on population structure and systematic relationships from morphology, isozymes, and chloroplast DNA. In: *Botany in the Curriculum: Integrating Research and Teaching.* Madison, WI: Botanical Society of America, 587.

SWOFFORD, D. L. 1999. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.* Sunderland, Massachusetts: Sinauer Associates.

THOMSON, J. A. 2000. New perspectives on taxonomic relationships in *Pteridium*. Pp. 15–34, in *Bracken Fern: Toxicity, Biology, and Control*, J. A. Taylor and R. T. Smith, (eds.) Leeds: International Bracken Group.

THOMSON, J. A. 2004. Towards a taxonomic revision of *Pteridium* (Dennstaedtiaceae). Telopea 10:793–803.

THOMSON, J. A., A. C. CHIKUNI and C. S. MCMASTER. 2005. The taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) from sub-Saharan Africa. Botanical Journal of the Linnean Society 148:311–321.

THOMSON, J. A., J. T. MICKEL and K. MEHLTRETER. 2008. Taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) of Laurasian affinity in Central and North America. Botanical Journal of the Linnean Society 157:1–17.

THOMSON, J. A., P. H. WESTON and M. K. TAN. 1995. A molecular approach to tracing major lineages in *Pteridium*. Pp. 21–28, in *Bracken: An Environmental Issue*, R. T. Smith and J. A. Taylor, (eds.) Aberystwyth: International Bracken Group.

TRYON, R. M. 1941. A revision of the genus *Pteridium*. Rhodora 43:1–31, 37–67.

TRYON, R. M. 1970. Development and evolution of fern floras of oceanic islands. Biotropica 2:76–84.

VARGAS, P., B. G. BALDWIN and L. CONSTANCE. 1998. Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). Proceedings of the National Academy of Sciences of the United States of America 95:235–240.

WILSON, J. T. 1963. A possible origin of the Hawaiian Islands. Canadian Journal of Physics 41:863–870.

WOLF, P. G. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. American Journal of Botany 84:1429–1440.

WOLF, P. G., E. SHEFFIELD, J. A. THOMSON and R. B. SINCLAIR. 1995. Bracken taxa in Britain: A molecular analysis. Pp. 16–20, in *Bracken: An Environmental Issue*, R. T. Smith and J. A. Taylor, (eds.) Aberystwyth: International Bracken Group.